



# COMBINED DECLARATION AND POWER OF ATTORNEY

part of  
paper # 5

As the below named inventor(s), I (we) hereby declare that:

My (Our) residence, post office address and citizenship(s) are as stated below next to my (our) name(s).

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

## HYDROGEN PRODUCTION USING HYDROGENASE-CONTAINING OXYGENIC PHOTOSYNTHETIC ORGANISMS. CONTINUATION OF PROVISIONAL APPLICATION SN 60/173,391, FILED DECEMBER 28, 1999

The specification of which (check one)

☐ is attached hereto ☒ as filed on December 22, 2000 as Serial No. 09/748,690  
and was amended \_\_\_\_\_.

I (We) hereby state that I (we) have reviewed and understand the contents of the above-identified specification, including claims, as amended by any amendment referred to above.

I (We) acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I (We) hereby claim foreign priority benefits under Title 35, United States Code § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

### PRIOR FOREIGN APPLICATION(S)

Priority  
claimed

☐ Yes ☐ No

Number	Country	Filed (Day/Month/Year)
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I (We) hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I (we) acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Serial No.: 60/173,391

Filing Date: 12/28/99

Status: Closed

**POWER OF ATTORNEY:** As the named inventor(s), I (we) hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Names and Registration Nos.	Names and Registration Nos.
Ken Richardson 27,378 Paul J. White 30,436	

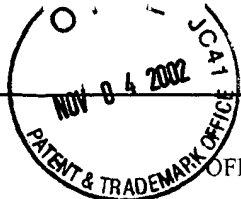
Send Correspondence to:

Paul J. White  
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(Name and Telephone Numbers)

Paul J. White  
303/384-7575

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U.S. DEPARTMENT OF ENERGY  
OFFICE OF ASSISTANT GENERAL COUNSEL FOR PATENTS  
RECORD OF INVENTION

NREL IR No.

99-29

This Record of Invention is an important legal document and proper care in its early and complete preparation will save important time and inconvenience in the future. The Instructions\* on the back should be read carefully before filling in the data.

A. Inventor: 1. Name(s): Anastasios Melis, Liping Zhang, John R. Benemann, Marc Forestier\*, Maria Ghirardi\*, and Michael Seibert\*

2. Title or Position: Professor (UC Berkeley), Postdoctoral Fellow (UC Berkeley), ~~Adjunct Professor~~ (UC Berkeley), Postdoctoral Fellow (NREL), Senior Scientist (NREL), and Principal Scientist (NREL)

Research Biochemist (UCB)

3. Employed by: University of California, Berkeley, and National Renewable Energy Laboratory\*

4. Permanent Address: 2745 Del Monte Ave., El Cerrito, CA 94530-1507; 1133 F 9th St., #26, Albany, CA 94710; 3434TiceCreek #1, Walnut Creek, CA 94595; 1890 Jellison St., Lakewood, CO 80215; 221 S. Zinnia Way, Lakewood, CO 80228; ; and 13134 W. Yale Pl., Lakewood CO 89228.

B. Title of Invention(1\*): ~~An Approach for Photo-Producing Large Amounts of Hydrogen Using Hydrogenase-Containing Oxygenic Photosynthetic Organisms~~

Photosynthetic Hydrogen Production by Microalgal Cultures

C. Description of Invention (2\*): Since hydrogenase-containing, oxygenic photosynthetic organisms (such as the green alga, *Chlamydomonas reinhardtii*) must be anaerobically adapted before they will produce hydrogen, current procedures call for treating algae in the dark with an inert gas atmosphere (no oxygen present) for up to four hours before the hydrogenase enzyme is activated and able to release hydrogen. Since hydrogen is generated mostly in the light and the organisms also produce oxygen in the light, hydrogen production is normally only a transient phenomenon. Shortly after hydrogen production commences, the oxygen concomitantly produced inactivates the hydrogenase enzyme and hydrogen production stops. We have observed that nutrient limitation (for example, sulfur deprivation) inhibits photosystem II function and eliminates net light-induced oxygen production by algae within 20-48 hours of nutrient deprivation of the growth medium. The key to this invention is the observation that shortly after this point, the culture will start to produce copious amounts of hydrogen in as little as 4-8 hours and for up to three days in the light if the culture vessel is sealed, and if the culture is not allowed to come in contact with atmospheric oxygen during the hydrogen-production stage. When hydrogen production stops, the culture can be regenerated by resupplying the missing nutrient (for example, sulfur as sulfate, etc.), and allowing, for example, 1:4 diluted cells to grow photosynthetically for about two doubling times. At that point the process can be repeated and more hydrogen can be produced. The hydrogen stream can be up to 87% hydrogen with a little nitrogen, traces of oxygen, and small amounts of carbon dioxide.

(See also UCB Invention & Technology Disclosure Form)

D. Dates and Places of Inventions:

1. Conception by Inventor (3\*) Summer 1997 (see also the attached chronology) At UC Berkeley

2. First Sketch or Drawing October 7-14, 1998 At NREL In Workbook 2352 Page 001-006

3. First Written Description October 7-14, 1998 At NREL In Workbook 2352 Page 001-006

4. Disclosure to Others (4\*) No external disclosure at this point (but see attached White paper) At \_\_\_\_\_

a. \_\_\_\_\_ 19 At \_\_\_\_\_

b. \_\_\_\_\_ 19 At \_\_\_\_\_

5. Completion of Model or Full Size Device December 28, 1998 to January 2, 1999 At NREL (Workbook 2352, pages 019-022)

6. First Test or Operation of Invention February 9-12, 1999 At NREL (Workbook 2403, pages 005-007)

E. Results of Tests and Extent of Use of Invention (5\*) In the first experiment we produced about 40 ml of hydrogen gas from 250 ml of culture and in the best experiment to date we produced at least 170 ml of hydrogen from 250 ml of culture in up to several days. Gas bubbles were produced and volume measurement were made by displacing water in a graduate cylinder.

F. Names of all persons having knowledge of facts stated under D and E: M. Forestier, M. Ghirardi, A. Melis, T. Flynn, P.-C. Maness, E. Greenbaum, ~~and~~ M. Seibert, JR Benemann and

Liping Zhang

G. Pertinent Reports (6\*): Hydrogen Program Weekly Highlight (an internal DOE document) sent to Neil Rossmessl and Sig Gronich on Dec. 2, 1998.

H. Other Closely Related Publications, Patents, and Patent Applications (7\*): This Record of Invention  
Wykoff et al. *Plant Physiology* 117 (1998) 129-139.  
Our previous NREL patents and patent applications

I. Rights of U.S. Government: ?

J. Licenses or Assignments: ?

K. Contracts Involved: Hydrogen Program contracts to NREL and the University of California, Berkeley

Contract No.: UCB-DOE Cooperative Agreement No. DE-FC36-98GO10278 (UC Berkeley)

Date:  
2/19/99

Subtask No.: HY913336 (NREL)

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Contractor and Address NREL		Type of Contract: ? Unclassified   Restricted   Confidential   Secret	
L. Signature of Witness:	Date:	Signature of Inventor(s): <i>[Signature]</i>	Date: 2/19/99
		<i>Maria Diana Jimenez H. Fordley</i>	2/28/99
Forwarded by (8*):			Date:

(OVER)

Rev. 11/8/93

Signature of Inventors (cont'd):

*Angelasio Melio*  
*John R. Jensen*  
*Liping Zhang*